IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the applications of:	)	Examiner: M. WOODWARD
ROGER P. EKINS	)	
Serial No.07/984,264	)	Group Art Unit: 1813
Filed: 1 December 1992	)	
For: DETERMINATION OF AMBIENT CONCENTRATION OF SEVERAL ANALYTES.	)	

DECLARATION OF DR JOHANN BERGER

Commissioner of Patents
and Trade Marks
Washington, DC 20231

Sir:

I, Dr Johann Berger, declare as follows:

1. I am a Director of New Technologies at Boehringer Mannheim GmbH, Tutzing, Germany, responsible inter alia for investigating and acquiring the rights to ideas for my company to develop. I have worked in the diagnostics field for 12 years. I have a good command of English. My curriculum vitae accompanies this declaration.

2. I am familiar with the content of the above patent application and the work of the inventor, Professor Roger Ekins in the field of immunoassay and, in particular, his "ambient analyte" methodology. I am also familiar with the objections raised by the Examiner in the Office Action of 23 August 1993. I have been asked to comment on the Examiner's objections that the above application would have been obvious to a person of ordinary skill in the art in view of Professor Ekins' earlier

application, WO84/1031 ('031), and Chang US 4591570 ('570).

3. I first met Professor Ekins at a meeting arranged in our office about 2 years ago. We were already aware of his work at that time, but were quite sceptical as to whether it would be applicable to the development of sensitive assays, since the notion of reducing the amount of binding agent in relation to the analyte to detect the analyte runs counter to the accepted idea that you need large amounts of binding agent to achieve high levels of analyte binding to gain maximum sensitivity. As a result of that meeting and subsequent discussions, we came to understand better the implications of the technology embodied in the above application (and Professor Ekins' other related patent applications) and realised in particular that it can allow highly sensitive assays to be carried out using very small amounts of binding agent.

4. It was our assessment that Professor Ekins' pioneering work had opened up an entire new approach in this field, allowing multi-analyte, miniaturised assays to be developed, which could be the basis for a new generation of assays, and that my company would have an excellent commercial opportunity if it developed them. We therefore negotiated an exclusive licence under all of Professor Ekins' patents and patent applications relating to the ambient analyte technology. At present, we are working on the commercial development of these assays and expect these to have important products on the market in the near future.

5. I therefore believe that a person of ordinary skill in

the art would find it surprising that the assay of the above application provides sensitivity enhancement by immobilising small amounts of binding agent (less than 0.1V/K moles) at high density in microspots. I believe it would not have been at all obvious to the person of ordinary skill in the art to do this to improve signal-to-noise ratios, because as mentioned above, this runs against the practice in the field using large amounts of binding agent to obtain optimal sensitivity. Further, I believe it is surprising that the sensitivity of an assay system increases as the size of the spot containing binding agent is reduced.

In example 2 of Ekins '031 the use of antibody having affinity constant of 2 x 10^{10} $lmol^{-1}$ (in an amount having a binding capacity of 10 fmoles) with samples of volume 0.2, 0.4 and 0.8mls, represents the use of V/K, 0.5V/K and 0.25V/K moles of binding agent. <u>These amounts are all in considerable excess of the more stringent 0.1V/K moles requirement of the above noted application</u>.

Even in light of Ekins '031, I do not think a person of ordinary skill in the art would have <u>further reduced</u> the amount of binding agent to the levels used in the above patent application, especially given the prejudice in the art against doing this and in the absence of any further suggestion or teaching.

6. Further, the assay of the present application has a further important advantage over '031. In the assay of '031, the person of ordinary skill in the art is taught to use a small amount of binding agent which only binds an insignificant

proportion of the analyte in the sample. This means that in '031, the assay is sample-volume independent. However, this reference requires knowledge of the expected concentration of the analyte so that the person of ordinary skill can determine what will constitute a small amount of binding agent in a given situation.

In contrast, the above application uses 0.1V/K moles of binding agent so that a small amount of analyte is removed from the total, irrespective of the analyte concentration. This represents a considerable practical advantage to the user of the assay, in particular where the expected concentration of an analyte varies over a large range. As '031 requires knowledge of the expected concentration of the analyte, it certainly does not suggest this advantage to the person skilled in the art.

7. Chang '570 concerns an assay in which immobilised antibodies are used to attempt to bind all the analyte in a sample, in this case red blood cells. In '570, multiple antibodies bind to each red blood cell, and so the value of the affinity constant of the binding agent for the analyte will be many times larger than suggested by the Examiner in the Office Action. Therefore, Chang uses an amount of binding agent much greater than 0.1V/K moles. This is not surprising as '570 represents a prior art assay of the type mentioned above.

8. As regards the calculation of 0.1V/K and 0.01V/K moles of binding agent discussed on pages 4 and 5 of the above application, I believe this calculation of the number of moles of binding agent could easily be made by the person of ordinary

skill in the art. Further, I believe this passage makes it clear that the subsequent calculation of the number of molecules of binding agent corresponding to these amounts merely an approximation. In fact, the number of molecules of binding agent is not an essential feature of the invention and the skilled person carrying out the assay does not need to know this to carry out the assay.

Similarly, the mention of 10^4 molecules in the above application is a preferred feature of the invention and would be recognised as such by those skilled in the art. Indeed the passage in question notes that even 10 molecules would be acceptable if an accuracy of 10% is acceptable to the user (see page 5 line 16).

9. In summary, I believe that nowhere does the prior art discussed above disclose using less than 0.1V/K moles of binding agent or the advantages this provides.

10. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001, Title 18 of the United States Code, and that such wilful false statements may jeopardize the validity of this application or any patent issued thereon.

Declared by .....
Dr. Johann Berger

Date Starnberg, den 22.11.1993.....

UR Nr. 3545 /K/1993 Pd/fl

Hiermit beglaubige ich die Echtheit umstehender, vor mir anerkannter Unterschrift von Herrn Dr. Johann B e r g e r, geb. am 23.12.1948, wohnhaft in 82327 Tutzing, Traubinger Str. 35, ausgewiesen durch seinen Führerschein.

Starnberg, den zweiundzwanzigsten November neunzehnhundertdreiundneunzig

Dr. Friedrich Kastenbauer
(N o t a r)